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Characterization of the interaction between Aβ 1–42 and glyceraldehyde phosphodehydrogenase

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Abstract: Advances in the understanding of AD pathogenesis have recently provided strong support for a modified Aβ protein cascade hypothesis, stating that several different Aβ assemblies contribute to the triggering of a complex pathological cascade leading to neurodegeneration. Both *in vitro* and *in vivo*, Aβ rapidly forms fibrils (fAβ), which are able to interact with various molecular partners, including proteins, lipids and proteoglycans. In a previous study aimed to identify some of these molecular partners of fAβ, we demonstrated that the GAPDH was specifically coprecipitated with fAβ. The aim of this study was to characterize this interaction. First, it was shown by TEM that synthetic GAPDH directly binds fAβ 1–42. Then rat synaptosomal proteins were purified and incubated with different forms of Aβ in various conditions, and the presence of GAPDH among the proteins coprecipitated with Aβ was studied by western blotting. Results showed that the interaction between GAPDH and fAβ 1–42 is nonionic, as is not impaired by increasing salt concentrations. GAPDH is coprecipitated not only by fAβ, but also by nonfibrillar forms of Aβ 1–42. The 41–42 Aβ sequence seems to be important in the interaction of GAPDH and Aβ, as more GAPDH was coprecipitated with fAβ 1–42 than with fAβ 1–40. GAPDH extracted from various subcellular fractions including mitochondria, was shown to interact with fAβ. Our data demonstrate a direct interaction between Aβ and GAPDH and support the possibility that this interaction has an important pathogenic role in AD. Copyright © 2008 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: beta-amyloid peptide; binding protein; GAPDH; coprecipitation assay; Alzheimer's disease

INTRODUCTION

Aβ peptide, which is overproduced in AD, accumulates extracellularly in neuritic plaques and aggregates intracellularly [1,2]. Aβ is a peptide of 39–43 amino acids, which is generated by multiple proteolytic processing of a large transmembrane precursor, the APP [3] and is present in different forms (monomers, oligomers, protofibrils and fibrils). By virtue of their structure and physicochemical properties, the different forms of Aβ are able to bind to a wide variety of biomolecules, including lipids, proteins and proteoglycans (for a review, see Refs 4 and 5). The binding of these molecules may interfere with various biological processes and it is well established that Aβ induces synaptotoxicity and neurotoxicity, directly and via an inflammatory response (for a review, see Refs 6–8). However, the molecular mechanisms of neurotoxic effects induced by the different forms and aggregation states of Aβ are still partly understood.

It was suggested that an intracellular Aβ processing pathway can be as pathogenic as that induced by Aβ released into the extracellular space [2,9]. Postulated steps of the extracellular Aβ processing pathway are the interaction of Aβ with multiple cell-surface receptors on neurons and microglia, thereby triggering signal transduction cascade that bring into caspase activation, free-radical generation and Ca^{2+} influx. An intracellular Ca^{2+} overload activates calpain proteases which trigger the activation of downstream caspases and the tau protein kinase Cdk5 (for a review, see Ref. 10). Alternatively, interactions with membrane lipids also induce toxicity, however by a distinct scenario (as reviewed in Ref. 5). In contrast, intracellular Aβ is predominantly liberated after APP

Abbreviations: Aβ, beta-amyloid; AD, Alzheimer's disease; APP, amyloid precursor protein; fAβ, fibrillar beta-amyloid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MC, mitochondrial (fraction); MS, microsomal (fraction); OD, optical density; PBS, phosphate buffer saline; SPM, synaptic plasma membrane (fraction); TEM, transmission electron microscopy.

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processing in the endoplasmic reticulum/intermediate compartment [11], the trans-Golgi network [12] and the endosomal–lysosomal system [13]. Moreover, Walsh *et al.* [14] has also provided evidence on the intracellular oligomerization of Aβ. Intraneuronal Aβ 1–42 has been linked to dysfunctions of the mitochondria [15,16], lysosomes and synapses, converging to cell death through a p53-mediated proapoptotic pathway. Decreasing brain metabolism is a significant cause of cognitive abnormalities in AD. Synapses are the first to show pathological symptoms in AD before onset of clinical symptoms. Because synaptic functions have high energy demands, interruption of energy supply could be an important factor in synaptic failure in AD. Mitochondrial dysfunction and disturbances might play an important role in neurologic diseases and chronic neurodegenerative conditions [17,18]. Collectively, substantial evidence has recently accumulated pointing to a combination of Aβ-induced intracellular and extracellular signaling pathways that determine neuronal dysfunction and neurodegeneration in AD [7].

In a previous work aimed at identifying new synaptosomal molecular partners of fAβ, we used a coprecipitation assay as a systematic approach, followed by mass spectrometric identification [19]. This study resulted in the identification of 40 proteins that specifically coprecipitated with fAβ 1–42, including the GAPDH [EC 1.2.1.12].

GAPDH is a key redox-sensitive enzyme in glycolysis that catalyzes the NAD-dependent conversion of the glyceraldehyde-3-phosphate to 1,3-diphospoglycerate. This protein is present in the cell in multiple isoforms, according to subcellular–nuclear or cytosolic–localization [20]. GAPDH has, apart from its glycolytic role, various membrane, cytoplasmic and nuclear functions, including membrane fusion, endocytosis, cytoplasmic mRNA regulation, microtubule bundling, nuclear tRNA export, DNA replication and DNA repair (for a review, see Ref. 21). Interestingly, a number of recent studies have shown that GAPDH plays an active role in various forms of apoptosis and may participate in neuronal death in AD, Parkinson's disease and Huntington's disease (for a review, see Refs 22 and 23). The different isoforms of GAPDH are differentially regulated during apoptosis, and seem therefore to have distinct apoptotic roles [24].

Because of the crucial importance of GAPDH, its interaction with fAβ could have a key role in AD, and needs to be further characterized. GAPDH has been reported to interact with the *C*-terminal region of APP but not with Aβ [25], whereas another study reported an interaction between monomeric Aβ and GAPDH [26]. The inconsistencies between these two studies have been attributed to different Aβ immobilization methods. Our previous work, that did not use immobilization method, could not allow to determine whether the coprecipitated GAPDH directly binds fAβ 1–42 or if it participates in a protein network interacting with fAβ 1–42. The present study aims to determine if there is a direct interaction between fAβ and GAPDH, and if so, to characterize this binding.

EXPERIMENTAL PROCEDURES

Materials

Aβ 1–40 and 1–42 (DEAFRHDSGYEVHHQKLVFFAEDVGSNK GAIIGLMVGGVV-IA) were synthesized in our laboratory on a solid phase support, as described in Ref. 27. After purification, Aβ was lyophilized twice from aqueous solution. fAβ was obtained by dissolving Aβ 1–40 or Aβ 1–42 in distilled water at 66 μM, and incubation for 48 h at 37 °C [19]. Nonfibrillar form of Aβ 1–42 was obtained by dissolving Aβ 1–42 immediately before use in distilled water at 66 μM, followed by centrifugation at 16 000 *g* for 30 min, and discarding the pellet.

β-crystalline (Sigma, St Louis, MO, USA) was dissolved in 10 mM Tris-HCl, pH 6.8, at 200 μM [19] and incubated for 48 h at 37 °C. Synthetic GAPDH (Sigma) was dissolved in distilled water at 40 mM.

TEM Analysis

The structure of the peptide aggregates (crystalline, Aβ 1–40, Aβ 1–42) was checked with TEM. Ten microliters of the aggregated peptide sample was adsorbed onto 400 mesh carbon-coated copper grids (Electron Microscopy Sciences, Washington, PA), fixed with 0.5% glutaraldehyde solution, washed 3x with water and finally stained with 2% uranyl acetate. Specimens were studied by using a Philips CM 10 transmission electron microscope at 100 kV. Images were taken with a MegaView II Soft Imaging System at a magnification of x 46 000.

Binding of synthetic GAPDH to fAβ 1–42 was studied by TEM after immunological labeling. Twenty micromolar of fAβ 1–42 and 20 μM of GAPDH or the same volume of water (40 μl, negative control) were incubated for 3 h, and the fibrils were precipitated by centrifugation for 30 min at 16 000 *g*. The pellet was resuspended in water and 10 μl of the mixture was adsorbed onto a nickel grid. The grid was saturated with PBS-milk, washed twice with incubation buffer (0.1% BSA, 0.1% NaN_3 in PBS), incubated for 5 min with anti-GAPDH (Chemicon, Temecula, CA) at 1 : 100, washed 3x in incubation buffer, incubated for 5 min in goat antimouse IgG labeled with gold particles of 5 nm (Electron Microscopy Science) at 1 : 10, washed 3x in incubation buffer, fixed with 0.5% (w/v) glutaraldehyde solution, washed 3x with water and finally stained with 2% (w/v) uranyl acetate. Specimens were studied as written above.

Purification of Rat Brain Subcellular Fractions

For coprecipitation experiments, proteins extracted from the SPM, MS or MC fractions were used. Subcellular fractionation of rat brains (4 Wistar males, 200–250 g) was performed as described in Ref. 28. Briefly, fresh forebrains were gently homogenized in 10 volumes of 10% sucrose, 0.05 mM $CaCl_2$, 0.5 mM dithiotreitol, 5 mM Tris-HCl, pH 7.4. The homogenates

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were centrifuged twice at 1000 *g*, and the combined supernatants were centrifuged at 20 000 *g* for 20 min. Pellets were suspended in 10% buffered sucrose and subjected to consecutive centrifugations at 20 000 *g* for 25 min and twice at 14 000 *g* for 20 min. The resulting synaptosomal pellets were lyzed by Dounce's homogenization and incubated for 30 min on ice in lysis buffer (0.05 mM $CaCl_2$, 0.5 mM dithiotreitol, 10% sucrose, 5 mM Tris-HCl, pH 8.1). The lysate was adjusted to 34% sucrose and fractionated on a 10/28.5/34% sucrose density step gradient spun at 128 000 *g* for 2 h. A highly enriched SPM fraction was obtained from the 28.5/34% interface, and the sedimented pellet contained the MC fraction. A crude MS fraction was obtained from the 12 000 *g* supernatant by consecutive centrifugations at 20 000 *g* for 25 min and 128 000 *g* for 1 h. The MS fraction was purified on a 10/28.5% sucrose step gradient centrifuged at 128 000 *g* for 2 h, the enriched MS fraction was collected at the 10/28.5% interface. SPM and MS fractions were diluted three-fold with 50 mM Tris-HCl pH 7.4 and pelleted at 128 000 *g* for 1 h.

Coprecipitation Binding Assays

This assay was performed with the method described in Ref. 29, modified slightly. The SPM, MS and MC pellets were resuspended in 4 ml of 1% Triton X-100 in pH 6.8 PBS containing protease inhibitor cocktail (Sigma), and centrifuged at 16 000 *g* for 30 min.

The supernatants containing the detergent-soluble proteins were incubated overnight at room temperature with the amyloid or with the crystalline (final concentrations: 20 μM Aβ, 20 μM crystalline).

1. To determine the nature of the binding of GAPDH to fAβ 1–42, SPM proteins were incubated with fAβ 1–42, adding increasing concentrations of NaCl (final concentrations: PBS containing 10, 100 and 200 mM NaCl).
2. To assess whether the interaction is specific to the fibrillar form of Aβ, a competitive assay was done, incubating the SPM proteins with 20 μM of the nonfibrillar and 20 μM of the fibrillar form of Aβ 1–42.
3. To determine sequence specificity of the binding, SPM proteins were incubated with the fAβ 1–40 or 1–42.
4. To assess the effect of GAPDH subcellular origin on its coprecipitation with Aβ, SPM, MS and MC fractions were incubated with the fAβ 1–42.

After the incubation, the samples were centrifuged at 16 000 *g* for 10 min, washed twice with 20 mM Hepes pH 7.0, and resuspended in 1% sodium azide in water. The protein concentration in the solution was evaluated using the BCA Protein Assay Kit (Novagen, Darmstadt, Germany), according to the supplier's instructions, using the fraction V of BSA (Sigma) as the standard.

Western Blot Analysis

Ten microgram of coprecipitated proteins were purified by 10% SDA-PAGE performed under reducing conditions, and the proteins were blotted onto a nitrocellulose membrane (Biorad, Hercules, Cal). The blot was saturated for 1 h with 5% (w/v) nonfat milk (Biorad) in PBS, washed with PBS and incubated overnight in the presence of mouse anti-GAPDH antibody (Chemicon) diluted 1 : 1000 in PBS. The membrane was then washed 3x with PBS, incubated for 1 h with a goat peroxidase-conjugated antimouse IgG antibody (DAKO, Glostrup, Denmark) diluted 1:10 000 in PBS, and washed 3x with PBS. The immune complexes were detected with chemo-luminescent substrate of peroxidase (Millipore, Billerica, MA, USA) according to the supplier's instructions. The images of immunoblotting were digitalized and the OD of each spot was quantified using the ImageJ software (http://rsb.info.nih.gov/ij/; [30]). Data were analyzed by the Mann–Whitney *U* test to compare two groups and the Kruskal–Wallis test to compare more groups, using the SPSS software. Differences with $p \leq 0.05$ were considered statistically significant.

RESULTS

Characterization of the Binding of GAPDH to fAβ 1–42

To determine if there is a direct binding between GAPDH and fAβ 1–42, synthetic GAPDH and fAβ fibrils were incubated together and, after washing, the GAPDH bound to the Aβ fibrils was immuno-stained using an anti-GAPDH gold-labeled antibody, then visualized by TEM (Figure 1(A)). Amyloid fibrils exhibited the morphology of fibrils characterized in other studies [19,31]. Antibodies bound to GAPDH appeared as round gold particles localized on the fibrils, whereas in negative control samples (fAβ and antibodies), only a few spots were nonspecifically stained (Figure 1(B)).

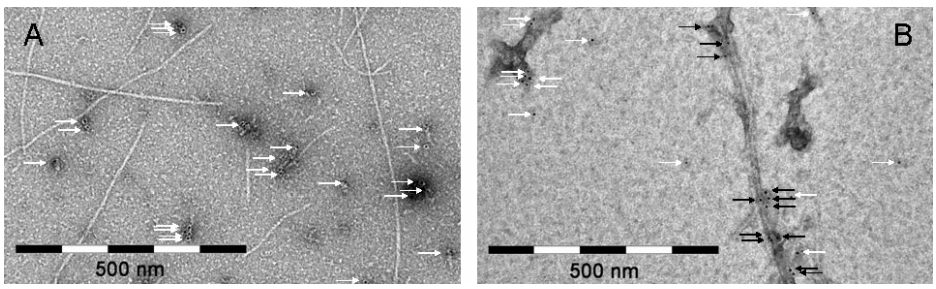

Figure 1 Immunolocalization of GAPDH on amyloid fibrils. GAPDH is stained with a gold-labeled antibody, and appears as round gold particles (dark spots) on the figure. (A): Negative control: only a few spots are nonspecifically stained (white arrow). (B): Incubation of fAβ 1–42 with synthetic GAPDH: the dark spots are localized on the fibrils (black arrow).

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The specificity of the interaction between GAPDH and fAβ 1–42 was evaluated after incubation of SPM GAPDH with fAβ 1–42 or with fibrillar crystalline (negative control). Western blot analysis showed that fAβ 1–42 coprecipitated more GAPDH than did fibrillar crystalline (Figure 2): the band of GAPDH precipitated with fAβ 1–42 had a significantly higher OD than the band of GAPDH precipitated with fibrillar crystalline ($Z_4 = 1.96$, $p = 0.05$).

Ionic interaction is a possible way of binding between proteins and Aβ, and is very sensitive to NaCl concentration. In order to determine whether the binding between GAPDH and Aβ is ionic, we coincubated the SPM fraction with varying NaCl concentrations. Results showed that the total quantity of the proteins coprecipitated with fAβ is inversely related to NaCl concentration, as measured with the BCA protein assay (Figure 3(A); $H_{4,8} = 10.39$, $p = 0.016$ for the four samples, (details in figure legend). In contrast, western blot analysis showed that the quantity of GAPDH coprecipitated with fAβ 1–42 does not noticeably change with increasing NaCl concentrations (Figure 3(B)), as the bands of GAPDH coprecipitated with fAβ 1–42 in the presence of increasing NaCl concentrations have similar ODs ($H_{3,9} = 1.87$, $p = 0.60$). These results indicate that the binding between fAβ 1–42 and GAPDH is nonionic.

Amyloid Aggregation State Specificity of the Interaction

To investigate whether GAPDH binds only to the fibrillar form of Aβ 1–42, a competitive coprecipitation assay

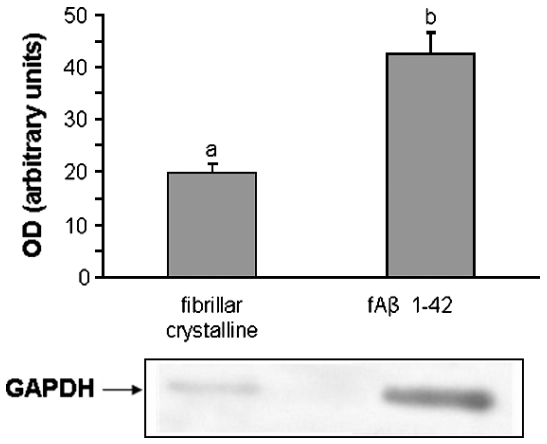

Figure 2 GAPDH specifically binds to fAβ 1–42. Synaptosomal membrane proteins were incubated with fibrillar crystalline or with fAβ 1–42. The presence of the GAPDH among the proteins coprecipitated with the fibrils was shown by western blot analysis. The band of GAPDH precipitated with fAβ 1–42 had a significantly higher OD than the band of GAPDH precipitated with fibrillar crystalline. Columns with different letters are significantly different ($p \leq 0.05$). Means (of four experiments), S.E.M. and representative examples of the bands (under respective columns) are shown.

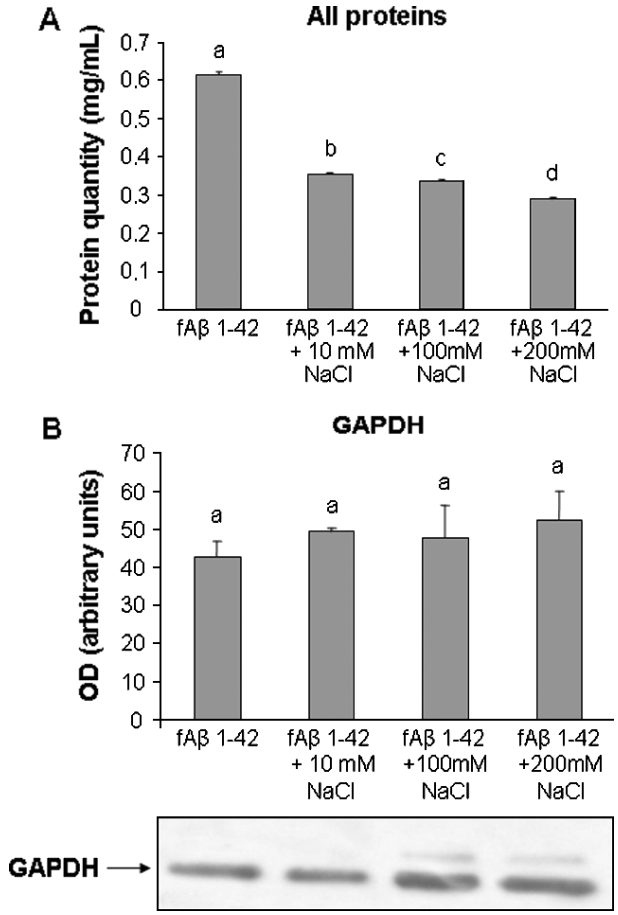

Figure 3 Increase of salt concentration decreases the total amount of proteins coprecipitated with fAβ 1–42, but does not have an effect on GAPDH coprecipitation with the fibrils. (A): Quantities (mg/ml) of synaptosomal membrane proteins coprecipitated with fAβ 1–42 in the presence of increasing salt concentrations. Means and S.E.M. are shown. (B): Immunostaining of GAPDH in the SPM proteins coprecipitated with fAβ 1–42 in the presence of varying salt concentration. Means (of four experiments), S.E.M. and representative examples of the bands (under respective columns) are shown. On each panel, columns with different letters are significantly different ($p \leq 0.05$).

was done using the SPM fraction and a mixture of fAβ 1–42 and a nonfibrillar form of Aβ 1–42. Western blot analysis showed that more GAPDH is coprecipitated with the competitive sample than with crystalline, but less GAPDH is coprecipitated with the competitive sample than with fAβ 1–42 (Figure 4; for the three samples, $H_{2,6} = 6.48$, $p = 0.039$; details are in figure legend). These results indicate that GAPDH binds also the nonfibrillar forms of Aβ 1–42.

Amyloid Sequence Specificity of the Interaction

To investigate whether the interaction between GAPDH and amyloid fibrils is specific to the final sequence of Aβ, SPM GAPDH was coprecipitated with either fAβ 1–40 or fAβ 1–42. Western blot analysis showed that fAβ 1–40

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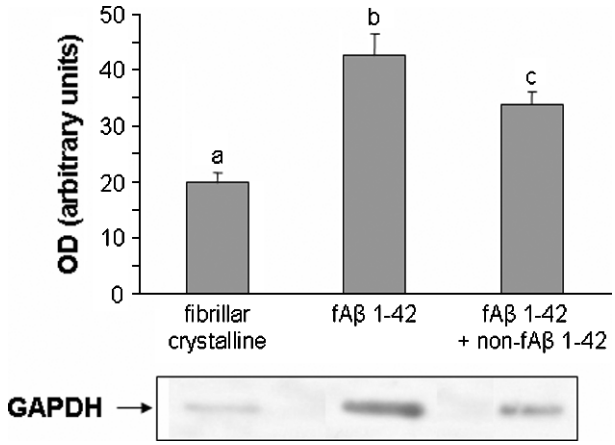

Figure 4 GAPDH binds the nonfibrillar form of Aβ 1–42. SPM proteins were coprecipitated with fibrillar crystalline, fAβ 1–42, and a mixture of fAβ 1–42 and nonfibrillar Aβ 1–42, then all the samples were immunoblotted with anti-GAPDH antibody. Densitometric analysis of Western blots showed that significantly less GAPDH is coprecipitated when nonfibrillar Aβ 1–42 is added to the sample, suggesting that GAPDH binds also this form of Aβ 1–42. Columns with different letters are significantly different ($p \leq 0.05$). Means (of four experiments), S.E.M. and representative examples of the bands (under respective columns) are shown.

does not coprecipitate significant amounts of GAPDH (Figure 5), as the band of GAPDH coprecipitated with fAβ 1–40 has an OD similar to the band of GAPDH coprecipitated with crystalline ($Z_5 = 1.77$, $p = 0.08$). Solutions of both Aβ forms contained fibrils as shown by TEM (data not shown).

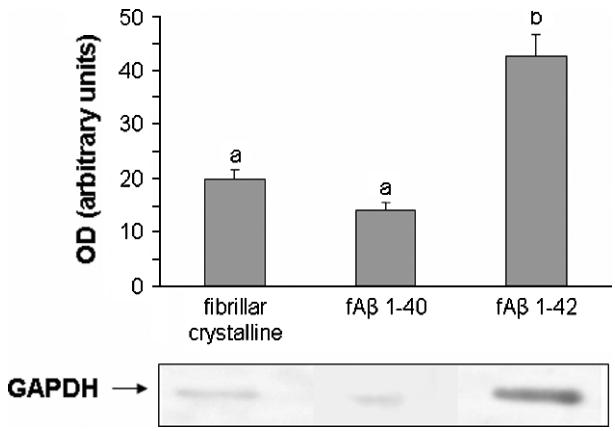

Figure 5 The interaction between Aβ and GAPDH is sequence specific. Synaptic plasma membrane proteins were coprecipitated with fibrillar crystalline, fAβ 1–42 or fAβ 1–40, then immunoblotted with anti-GAPDH antibody. Considerably less GAPDH is coprecipitated with the Aβ 1–40 fibrils than with the Aβ 1–42 fibrils. Columns with different letters are significantly different ($p \leq 0.05$). Means (of four experiments), S.E.M. and representative examples of the bands (under respective columns) are shown.

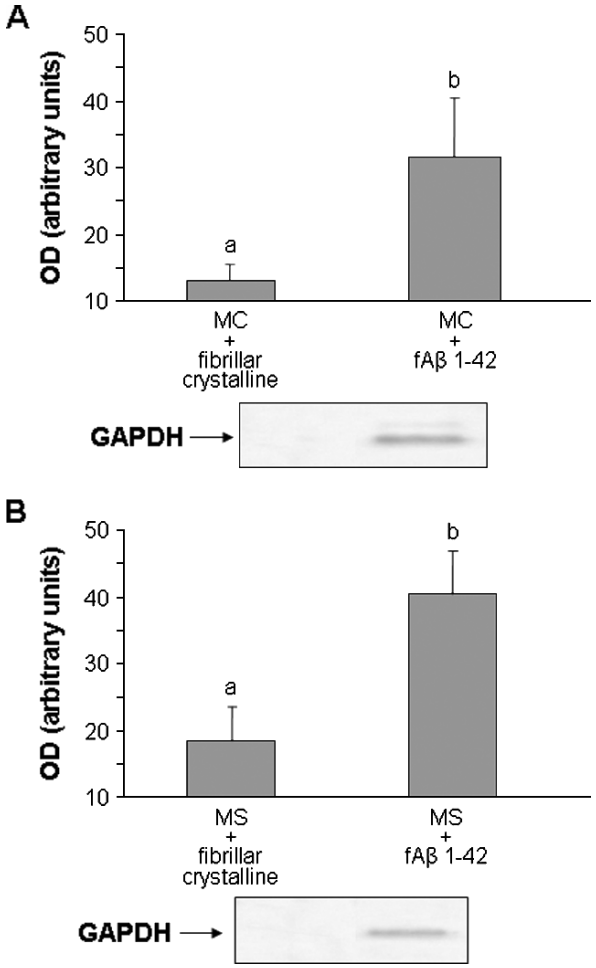

Figure 6 Fibrillar Aβ 1–42 coprecipitates GAPDH extracted from MC and MS fractions. Proteins extracted from the MC and MS fractions were coprecipitated with fibrillar crystalline or fAβ 1–42 and immunoblotted with anti-GAPDH antibody. (A): Western blot analysis showed that the band of MC GAPDH coprecipitated with fAβ 1–42 has a higher OD than MC GAPDH coprecipitated with crystalline. (B): Similarly, the band of MS GAPDH coprecipitated with fAβ 1–42 has a higher OD than MS GAPDH coprecipitated with crystalline. On each panel, columns with different letters are significantly different ($p \leq 0.05$). Means (of four experiments), S.E.M. and representative examples of the bands (under respective columns) are shown.

Subcellular Origin of the GAPDH Precipitated with Aβ

As GAPDH has different isoforms according to its subcellular localization, we aimed to determine whether these coprecipitate differentially with fAβ 1–42. In order to do that, proteins extracted from the MC and MS fractions of rat brain were incubated with fAβ 1–42, and the proteins coprecipitated with the amyloid fibrils were analyzed by western blotting. The GAPDH extracted from each of the MS and the MC fractions was coprecipitated with fAβ 1–42, and not or very little with the crystalline used as negative control (Figure 6; MC fraction: $Z_5 = 2.12$, $p = 0.034$; MS fraction: $Z_6 = 2.02$,

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$p = 0.04$). Therefore, the GAPDH forms found in the SPM, MS and MC fractions all bind to fAβ 1–42.

DISCUSSION

According to the amyloid cascade hypothesis (reviewed in Ref. 7), the Aβ peptide plays a major role in the etiology of AD. The details of its mechanism of action remain, however, largely unknown, partly because of its potential effects on a wide variety of biomolecules that it has been shown to bind (for a review, see Ref. 5). One of the most important and ubiquitous molecules that may mediate the neurotoxic action of Aβ is GAPDH, as we have recently shown – by two different mass spectrometric methods – that it is coprecipitated with fAβ [19]. Moreover, other studies have suggested an interaction of GAPDH with monomeric Aβ [26]. The present study aimed to characterize the interaction between fAβ and GAPDH.

Previous results showing coprecipitation of GAPDH in the presence of fAβ 1–42 can be due to several mechanisms. The simplest possibility is a direct binding between GAPDH and fAβ 1–42, however GAPDH could also participates in a protein network interacting with fAβ 1–42. Alternatively, GAPDH could be precipitated by Aβ because Aβ promotes disulfide bonding and aggregation of GAPDH in the cell [32]. Our TEM experiments show very close physical proximity of GAPDH and fAβ 1–42, pointing to a direct interaction between these molecules.

In order to assess whether the interaction between GAPDH and fAβ 1–42 is ionic – as it is the case between the CLAC protein and fAβ 1–42 [33] – we studied the effect of the addition of salt on their binding. Our results show that coprecipitation of GAPDH and fAβ 1–42 is similar in solutions containing different concentrations of salt, suggesting that their interaction is not of an ionic nature.

Although the present study focused mainly on the interaction between GAPDH and the fibrillar form of Aβ, we also evaluated whether GAPDH binds to nonfibrillar Aβ. In order to avoid potential bias induced by chemical stabilization of the nonfibrillar forms of Aβ, no cross-linking was done. Considering the kinetics of Aβ oligomerization and fibrillization [34], this mixure should contain Aβ at various oligomerization and fibrillization states. A coprecipitation assay showed that there is indeed an interaction between GAPDH and nonfibrillar Aβ, consistent with other studies showing that GAPDH can bind to monomeric Aβ [26]. This result is one of the most promising of this work, as intraneuronal, nonfibrillar forms of Aβ have been implicated in neuronal failures (for a review see Ref. 8).

As GAPDH does not interact with the fibrillar structure *per se*, we investigated the effect of the two extra residues in Aβ 1–42 on Aβ binding to GAPDH. Our results show that contrary to Aβ 1–42, Aβ 1–40 does not coprecipitate a significant amount of GAPDH. This suggests that the final sequence of Aβ may be important in this interaction directly or via an increase in the Aβ fibrillation rate. Indeed, Aβ 1–42 is more prone than Aβ 1–40 to form fibrils [35–37], and only the fibrillar form of Aβ coprecipitates GAPDH, although both the fibrillar and nonfibrillar forms bind GAPDH. However, as the Aβ 1–40 solution contained fibrils, the complete absence of GAPDH coprecipitated with Aβ 1–40 points rather to a direct influence of the final Aβ sequence on its affinity with GAPDH than to indirect effects on fibrillation rate. The critical importance of the final Aβ sequence on its binding with other proteins has already been reported, for example, for the CLAC protein. Contrary to GAPDH, the CLAC protein binds both fAβ 1–42 and fAβ 1–40 but not the nonfibrillar form of Aβ 1–42, suggesting a distinct mechanism, probably ionic [33].

GAPDH of different subcellular origin might not bind fAβ 1–42 with the same affinity, as some binding sites could be masqued by an interaction with subcellular specific molecular partners, therefore we evaluated the interaction between fAβ 1–42 and GAPDH extracted from various fractions of the rat brain. We extracted GAPDH from fractions where GAPDH has already been found by other studies, including synaptosomes [38], microsomes [39] and mitochondria [40]. Our results show that fAβ 1–42 coprecipitates with GAPDH extracted from the SPM fraction, as well as the MC and the MS fractions, suggesting that Aβ can affect GAPDH activity in any of those subcellular locations. The fact that intracellular Aβ can effectively access mitochondria and alter its function in AD [41] points to the potential importance of mitochondrial actions of Aβ. It is also interesting to note that qualitative alterations of GAPDH have been observed in specific subcellular fractions of AD brains [42].

GAPDH has been associated with various neurodegenerative disorders. It has been shown to bind proteins implicated in neuronal diseases, e.g. huntingtin [43], APP [25], it is present in neurofibrillar tangles [44] and is colocalized with α-synuclein in Lewy bodies [45]. GAPDH may function as a proapoptotic protein in neuronal cells [46]. Moreover, it has a specifically reduced activity in animal models of AD [47] as well as in Huntington's disease cells [20,42,48].

Overall, our data confirm that Aβ can directly interact with GAPDH in the brain. Such an interaction could have a crucial importance in the etiopathology of AD, as Aβ-GAPDH interactions, altering GAPDH structure *in vivo*, may affect energy generation and a variety of fundamental cellular pathways in AD cells. GAPDH could mediate the neurotoxic activity of Aβ in AD by several mechanisms, which are outlined below. (i) Increased levels of Aβ in AD may decrease the glycolytic activity of GAPDH (e.g. by increasing disulfide bonding or by inducing oxidation of

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GAPDH), [49], causing a significant reduction of cellular ATP production, contributing to a neurodegenerative process [22,47]. A role of GAPDH dysfunction in AD is supported by the special importance of glycolysis in neurons because of their high energy needs and the fact that a reduction in glucose metabolism precedes cognitive dysfunction and is therefore believed to be an important early event in AD [50,51]. (ii) Binding between GAPDH and Aβ may affect not only energy production but also other, nonglycolytic functions of GAPDH, such as membrane fusion, endocytosis, cytoplasmic mRNA regulation, microtubule bundling, nuclear tRNA export, DNA replication and repair (reviewedin Ref. 20). According to our results, GAPDH can bind both fibrillar and nonfibrillar forms of Aβ, therefore the effects of Aβ on glycolytic or nonglycolytic enzymatic activity of GAPDH may be due to either or both Aβ forms. (iii) Finally, as the interaction of GAPDH with mutant α-synuclein has been implicated in abnormal autophagic processes [52], and autophagy seems to be an important mechanism in AD [53], the consequences of the GAPDH–fAβ interaction on autophagic processes should be further explored.

Our data demonstrate a direct interaction between Aβ and GAPDH and support the possibility that this interaction has an important pathogenic role in AD. Ultimately, the binding of Aß with GAPDH may provide a new therapeutic target in AD.

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